



EXPRESS MAIL NO: EL913520123US  
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Lars Bjorck and Magnus Rassmussen  
Application No. : 09/847,539  
Filed : May 1, 2001  
For : STREPTOCOCCAL ALPHA ZM BINDING PROTEIN

Art Unit : 1642  
Docket No. : 100084.415US  
Date : November 27, 2001

Box Missing Parts  
Commissioner for Patents  
2900 Crystal Drive  
Arlington, VA 22202-3513

PRELIMINARY AMENDMENT REGARDING SEQUENCE DISCLOSURES

Commissioner for Patents:

In response to the Notice to File Missing Parts dated June 4, 2001, please amend the above-identified application as follows:

In the Specification:

Please delete the section of the application entitled "Sequence Listing" at the end of the specification and insert the enclosed Sequence Listing therefor.

Please replace the paragraph beginning at page 3, line 10, with the following rewritten paragraph:

Fig 2. A schematic comparison between protein GRAB and protein G is shown in A. The complete nucleotide (SEQ ID NO: 27) and amino acid sequence (SEQ ID NO: 7) of *grab*/protein GRAB is shown in B.

Please replace the paragraph beginning at page 26, line 11, with the following rewritten paragraph:

Hybridization protocol is carried out as follow. Streptococci were grown in Todd-Hewitt broth with 0.2% yeast extract (THY) in 5% CO<sub>2</sub> at 37°C. Genomic DNA was prepared from *S.pyogenes*. 20µg of DNA was cleaved by *EcoRI* and subjected to agarose gel electrophoresis and capillary blotting onto Hybond-N filters (Amersham, Amersham, UK). A probe was generated by PCR using *Taq* polymerase and synthetic oligonucleotides with sequences GACTCACCTATCGAACAGCCTCG (SEQ ID NO: 28) and AGCTTCTTCTGATTGTAAAGCG (SEQ ID NO: 29) hybridizing to *grab*. The PCR product was purified on a MicroSpin<sup>TM</sup> S-200 HR column and was radiolabeled with [ $\alpha$ -32P]dATP using bacteriophage T4 polymerase. Membrane was prehybridized in a solution of 6xSSC, 0.5% SDS, 5xDenharts solution, and 100µg/ml salmon sperm DNA at 50°C for two hours. Probe was boiled for five minutes and added to a solution of 6xSSC, 0.5% SDS and 5xDenharts solution and incubated for 14 hours at 65°C. This was followed by washing at room temperature in 2xSSC +0.5% SDS for five minutes and 2xSSC+0.1% SDS for 15 minutes. Further washes were performed in 0.1xSSC+0.5% SDS at 37°C for one hour and in 0.1xSSC+0.1% SDS at 53°C for 30 minutes. Filter was air dried followed by exposure on BAS-III imaging plate and scanning with Bio-Imaging Analyser BAS-2000.

Please replace the paragraph beginning at page 31, line 25, with the following rewritten paragraph:

For an inhibition ELISA, post immune sera from sheep immunized with the peptide conjugates mentioned above, were pre-incubated at 37°C, for 1 hour, at a dilution of 1/10,000, with the corresponding free peptide at concentrations ranging for 0 to 10µg/ml. For controls, post

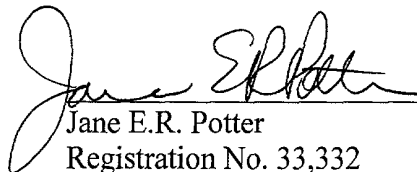
immune sera (1/10,000) were incubated with Spy-PH-QKQ19 (10µg/ml). This peptide has the sequence QKQQQLETEKQISEASRKS C\*-COOH (SEQ ID NO: 30). The serum peptide mixtures were then assayed on GRAB coated plates, as previously indicated.

### REMARKS

The enclosed electronic and paper copies of the Sequence Listing include no new matter that goes beyond the original application as filed, but are supplied to fulfill the requirements as outlined in the Notice to File Missing Parts. Furthermore, the above amendments, which merely direct the insertion of the Sequence Listing and insertion of sequence identifiers, include no matter that goes beyond the original application as filed. Applicants respectfully submit that the above-identified application is now in compliance with 37 C.F.R. §§ 1.821-1.825.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The first of the attached pages is captioned "Version with Markings to Show Changes Made."

Respectfully submitted,  
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# VERSION WITH MARKINGS TO SHOW CHANGES MADE

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Paragraph beginning at page 3, line 10, has been amended as follows:

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